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CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

Volume 33, No. 3 September 2004

CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society
www.carnivorousplants.org

Volume 33, Number 3
September 2004

Front Cover: Anthocyanin-free *Sarracenia alata*. Photograph by Carl Mazur. See article on page 76.

Back Cover: A large flowered form of *U. bisquamata* in the wild near Hermanus. Photograph by Robert Gibson. Article on page 77.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Contributors should review the "Instructions to Authors" printed in the March issue of each year. Advertisers should contact the editors.Views expressed in this publication are those of the authors, not the editorial staff.

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Date of effective publication of the June 2004 issue of Carnivorous Plant Newsletter: 9 June 2004.

The ICPS is the International Cultivar Registration Authority (ICRA) for cultivated carnivorous plants according to The International Code For The Nomenclature of Cultivated Plants. Send relevant correspondence to the ICPS, Inc.

PUBLISHER: ICPS, Inc., Pinole, California. Published quarterly with one volume annually. Desktop Publishing: Steve Baker, 5612 Creek Point Drive, Hickory, NC 28601. Printer: Kandid Litho. Logo and masthead art: Paul Milauskas. Dues: $25.00 annually. © 2004 Carnivorous Plant Newsletter. All rights reserved. ISSN #0190-9215.

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Pinguicula crystallina Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid (Lentibulariaceae) in Calabria (Southern Italy). Cytotaxonomical study and *ex situ* conservation in the Botanic Garden of Calabria University

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Keywords: Ecology: conservation, cultivation, genetics — *Pinguicula crystallina*, *Pinguicula hirtiflora*.

Introduction

According to Casper (1966), *Pinguicula hirtiflora* Ten. (Lentibulariaceae) belongs to the subgenus *Isoloba* Barnhart, section *Cardiophyllum* Casper. It was originally described from plants found in the area of Castellammare di Stabia (Salerno, Southern Italy; Tenore, 1811).

Plants corresponding to this original description occur in Italy in Campania and Calabria (Casper, 1962; Casper, 1970; Pignatti, 1982; Moraldo *et al.*, 1986; Pinto *et al.*, 2000); the citation with doubts (Casper, 1962) for plants in Abruzzi (Mount Pelone) were later shown to be *P. vulgaris* L. (Tammaro & Pace, 1987). *Pinguicula hirtiflora* populations are found at locations from southeastern Italy to the southwestern part of the Balkan Peninsula (Casper, 1966). Moreover this unit was recently reported, with several doubts on its spontaneity, for France in the Roya river Valley (Joly et al., 2002).

It is the only Italian *Pinguicula* which has a tropical growth type (Casper, 1962; Casper, 1966; Legendre, 2000), i.e. it shows an overwintering basal rosette that makes it interpretable as a Thermophilous relic. In contrast, other *Pinguicula* species from Italy overwinter as buds (hibernacula), and they are all microtherm elements. *Pinguicula hirtiflora* has recently been reduced to a subspecies (Strid, 1991) of *P. crystallina* Sibth. et Smith, this latter taxon being a vicarious oriental unit that occurs from Cyprus to Anatolia (Casper, 1970; Greuter *et al.*, 1989).

Pinguicula hirtiflora is very rare and localized in Italy, justifying its IUCN listing as "Vulnerable" (VU; Conti *et al.*, 1997). In Calabria, where it is known from only one stand, it is assigned to the category "Critically Endangered" (CR). These plants are at approximately 200 m a.s.l. on stillicidious calcareous cliffs along the Celadi stream. The stand is subject to great risk because it is near a road, and the population is only a few hundred individuals in an area of around 50×10 meters. Recent road maintenance has destroyed part of the population. We have therefore placed plants into cultivation at the Botanical garden of the University of Calabria, with the goal of preserving the plant *ex situ*. The presence of *P. hirtiflora* in the Botanical Garden has allowed us to conduct a karyological study that has given interesting results.

Material and methods

Approximately 20 plants were collected in October 2000 and placed into greenhouse cultivation. The microhabitat built for them consists of a calcareous, friable substratum, over which water continuously drips. Plants that occur with *P. hirtiflora* (liverworts, Marcantiophyta; ferns, *Adiantum capillus-veneris* L.) are grown on the substratum so as to reproduce the conditions of the natural habitat as accurately as possible (see Figure 3).

Pinguicula hirtiflora herbarium specimens in Florence (FI), Naples (NAP), and Rome (RO) were examined in order to make morphological and chorological observations and comparisons to the plants from Calabria.

Specimina visa - Italy, Campania: Locis arenosis "aqua santa" in regione superiore M. S. Angelo prope Castellammare regionis neapolitanese, 3/VI/1874, *Henker* (FI); rupi umide all'Acquasanta di Monte Sant'Angelo di Stabbia, 22/VIII/1891, *Martelli* (FI); Amalfi: Valle delle Ferriere, 31/V/1952, *Moggi* (FI); ad fontium stillicidis Acqua Santa M.te S. Angelo di Castellammare, s.d., *Tenore* (FI); Castellammare Acqua Santa, IX/1843, *Avellino* (FI); adorna bellamente le stalattiti della Grotta Bonné, 10/V/1862, *Pasquale* (FI); Ad rupes humidas Aqua S.ta montis S. Angelo supra Castellammare, 2/VIII/1855, *Huet du Pavillon* (FI); Grotta dei Porci ossia Grotta dell'Acquasanta M. S. Angelo di Castellammare ossia dei Tre Pizzi 1200 m ca., 22/VIII/1891, *Sommier* (FI); M. S. Angelo di Castellammare rupi stillanti all'acquasanta 1400 m ca., VII/1911, *Guadagno* (FI); M. S. Angelo di Castellammare. Rupi calcaree acquitrinose 1250-1800 m, 27/VI/1909, *Pellanda* (FI); Vietri a mare (Salerno) nuova stazione sul livello del mare scoperta da Hoffman, su rocce calcaree del Trias stillanti, 4/X/1954, *Alexander* (FI); Napoli, 1943, *Alexander* (FI); in paludosi Amalfi, 1876, *Lacaita* (FI); Faggeti in uno stillicidio all'Acqua Santa circa 1300 m presso Vico equense (M. S. Angelo napoletano), 24/VIII/1891, *Biondi* (FI); Ravello (Salerno) calcare 350-400 m, 28-29/VII/1921, *Fiori* (FI); Valle della Ferriera (Amalfi), 31/V/1952, *Corradi, Bavazzano et Contardo* (FI); Castellammare, VI, *s.l.* (RO); Montibus Stabiarum, s.d., *s.l.* (RO); Monti Stabiani, VI/1879, *Pasquale* (RO); Sulla roccia per la quale gronda l'acqua così detta Santa alla base del pianalto primo di S. Michele a Castellammare, 1883, *Pedicino* (RO); In unico loco prope Neapolim all'Acqua Santa M. S. Angelo a Castellammare, s.d., *Pasquale* (RO); A aquae stillicidiis Acqua Santa supra M.te S. Angelo, 3/VII/1877, *Pasquale* (RO); All'Acqua Santa sul M.te S. Angelo di Castellammare, s.d., *Tenore* (RO); Monte S. Angelo di Castellammare, VII/1895, *Honig* (RO); Amalfi, s.d., *s.l.* (RO, sub *Pinguicula vulgaris*); Grotta di Bonnée, sotto la Trinità, VI/1864, *s.l.* (RO); Grotta di Bonnée a Cava, VI/1868, *Pedicino* (RO); Grotta di Bonnée sotto la Trinità di Cava ai Mulini, VI/1864, *Pedicino* (RO); Avvocatella sopra Cava, e presso il Mulino della Trinità, s.d., *Tenore* (NAP); Castellammare, s.d., *Tenore* (NAP); Calabria: Presso Rossano, Torrente Celadi, alt. ca. 200 m s.l.m., 20/IV/1979, *Del Prete, Garbari, Cesca* (CLU); Greece: In valle laterali sinistra fluminis Achellòos vico Petrotòn 2 Km orientem versus (Grecia, Thessalia, Nomos et Eparchia karditsis) alt. 500 m. s.l.m., in rupibus schistosis scaturiginosis Adianto capillus veneris admixto, 19/IX/1980, *Binder, Hagemann, Hempel et Raus* (FI); In Monte Parnasso in scaturiginis reg. abietinae, VIII/1852, *Heldreich* (FI); In saxosis irriguis reg. super. M. Olympus Thessaliae, 21/VII/1857, *Heldreich* (FI); Karistae greola nächst Vucia (Flora for Hercegovina), 2/VI/1872, *Pantocsek* (FI, sub *Pinguicula laeta* mihi); Olympus Thessalia ad scaturigines prope Hogios Dionyous, 12/IX/1910, *Halàcsy* (FI); In Monte Korax Aetoliae objectae. In regione abietina superiori alt. 5500 fts., 24/VII/1879, *Heldreich* (FI); 5500 fts., west of Katara, north-facing slope in dense shade of beech-trees sopping-wet bank of stony alluvium and leaf mould by streams and with water percolating through it; exquisite lavender flowers; 3-4 m., 6/VIII/1964, *Archibald* (FI); Habitat in regione superiori montes Chelmos Peloponesi (Apzanua Opu) prope Stygem alt. 6000 fts., 6/VII/1851, *Orphanides* (FI, sub *Pinguicula megaspilaea* Boiss. et Orphanides).

For the karyological study, root tips were treated with a 0.3% solution of colchicine and fixed in Carnoy; afterwards they were hydrolyzed in 1N HCl solution and coloured with fuchsine; finally, they were squeezed in acetic orcein for counting and observation of chromosomes. In some cases we used phase contrast. At least five plants were used in order to establish the chromosome number.

Systematic study

Pinguicula crystallina Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid, in Strid & Tan, Mountain Flora of Greece 2: 276 (1991).

Basionym: *Pinguicula hirtiflora* Ten., Fl. Napol. 1: 6 (1811).

Lectotypus (here designated, Figure 1): M.te della Cava di Castellammare, all'acqua Santa, s.d., *Tenore* (NAP!, erroneously designated as the holotype by Casper, 1966); other original material: Avvocatella sopra Cava, e presso il Mulino della Trinità, s.d., *Tenore* (NAP); Castellammare, s.d., *Tenore* (NAP); ad fontium stillicidis Acqua Santa M.te S. Angelo di Castellammare, s.d., *Tenore* (FI); All'Acqua Santa sul M.te S. Angelo di Castellammare, s.d., *Tenore* (RO).

Synonyms (Casper, 1966; Strid, 1991): *P. vulgaris* Petagna non L., Inst. Bot. 2: 38 (1787), nom. illeg., *P. albanica* Griseb., Spicil. Fl. Rumel. 2: 9 (1844); *P. laeta* Pant., Österr. Bot. Z. 23: 80 (1873); *P. megaspilaea* Boiss. et Orphanides in Boiss., Fl. Or. 4: 2 (1875), nom. inval.; *P. hirtiflora* Ten. var. *megaspilaea* (Boiss. et Heldr.) Schindler, Österr. Bot. Z. 58: 62 (1908); *P. vulgaris* L. var. *hirtiflora*

Figure 1. Lectotype of *Pinguicula hirtiflora* Ten., conserved in NAP.

(Ten.) Fiori, N. Fl. An. Ital. 2: 472 (1925); *P. louisii* Markgraf, Ber. Deutsch. Bot. Ges. 44: 430 (1926); *P. hirtiflora* Ten. var. *decipiens* Bornmüller, Ungar. Bot. Bl. 32: 129 (1933); *P. hirtiflora* Ten. var. *euboea* Beauverd et Topali, Bull. Soc. Bot. Genève sér. 2, 28: 155 (1938); *P. hirtiflora* Ten. var. *louisii* (Markgraf) Ernst, Bot. Jahrb. Syst. 80: 186 (1961).

In addition to the taxa listed above, we believe the following to by synonymic: *P. hirtiflora* Ten. f. *pallida* Casper, Biblioth. Bot. 127/128: 107 (1966); *P. hirtiflora* Ten. var. *gionae* Contandriopoulos et Quezel, Rev. Biol. Ecol. Medit. 1: 30 (1974).

The studied Calabrian plants have a peculiar chromosome number, $2n = 27$ (Figure 2), a number that was also reported for this unit in material from Thessalia, Greece (Strid & Franzen, 1981). For plants collected in the same Calabrian loeality of our ones, the chromosome complement $2n = 28$ (Mikeladze & Casper, 1997) was already reported. Populations from the locus classicus in Campania have diploid chromosome complements $2n = 16$ (Honsell, 1959). Contandriopoulos & Quezel (1974) reported $2n = 16, 24, 32, 48$ for this unit in Greek material. They tried to correlate the ploidy level with some minor morphological differences, and identified four varieties for *P. hirtiflora*. We think the chromosome numbers $2n = 27, 28$ to be strictly correlated to triploids $2n = 24$ by ascending aneuploidy phenomena (or perhaps occurrence of B chromosomes, although it is very difficult to establish it for the extreme homogeneity of the chromosome size). A situation, in our opinion, to be interpreted in a similar way, occurs in *P. crystallina* subsp. *crystallina*: Contandriopoulos & Quezel (1974) counted $2n = 24$ chromosomes in material from Turkey, Mikeladze & Casper (1997) counted $2n = 28$ chromosomes in plants from Cyprus (where the species was described).

We do not pretend, in this work, to give an exhaustive survey of the variability of *P. crystallina* subsp. *hirtiflora*, since we have not been able to examine specimens from some places (i. e. Albania); however our plants are fully identifiable with typus material, and the quantitative differences observed, between Greek and Italian material are weak (Table 1); thus, to the actual state of knowledge, in our opinion the differences (both morphological and karyological) do not allow us to distinguish (for nomenclatural purposes) taxonomic categories within *P. crystallina* subsp. *hirtiflora*; although under this binomial probably more than one taxa are united.

Table 1. Morphological and karyological features of *Pinguicula crystallina* Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid from different provenances. Data come from bibliography, herbarium specimens cited in the text and the present study. Values are in 10-90 percentiles with extreme values in brackets.

	Italy, Calabria	Italy, Campania	Greece
length of leaves	2-4 cm	2-4(6) cm	(3)5-6 cm
width of leaves	10-15 mm	10-18 mm	15-25 mm
length of scape	7-10 cm	(3)4-7(10) cm	10-12 cm
number of scapes	1-3	(1)2-4(7)	1-4
length of corolla	13-14 mm	10(15) mm	10-15 mm
length of upper lobes	5 mm	(4)5-6(7) mm	4-5 mm
length of lower lobes	8 mm	(4)7-8(12) mm	6 mm
length of spur	8-9 mm	6(10) mm	10-15 mm
length of calyx	3 mm	2-3(4) mm	3-4 mm
chromosome number	$2n=27$[a], 28[b]	$2n=16$[c]	$2n=16, 24, 27, 32, 48$[d]

[a]See Figure 2.
[b]Mikeladze & Casper, 1997.
[c]Honsell, 1959.
[d]Contandriopoulos & Quezel, 1974; Strid & Franzen, 1981.

Conservation *ex situ*

After almost two years the plants have fully adapted to artificial cultivation. Flowers are produced plentifully April-July (Figures 4,5), resulting in seedlings (Figure 6). Vegetative reproduction has not

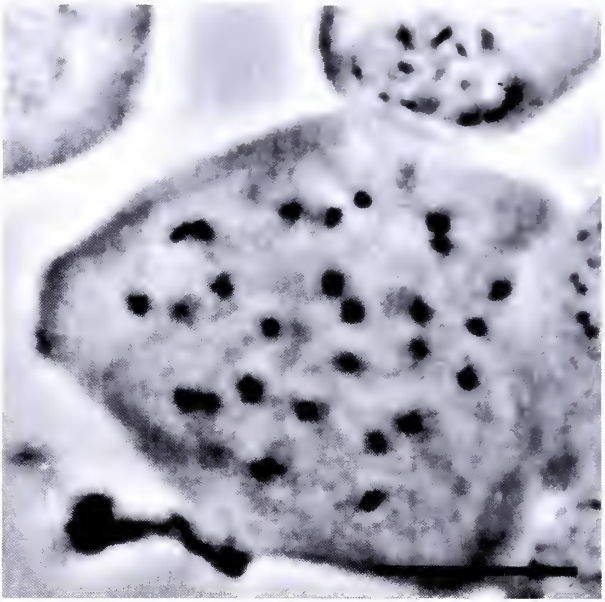

Figure 2. Metaphasic plate in root tip of *P. crystallina* subsp. *hirtiflora* from Rossano (Calabria, Southern Italy), showing $2n = 27$ chromosomes. Scale bar = 5µm

been observed for this plant. The reproduction by seed is unexpected because of the anomalous diploid chromosome number, so we think that the phenomenon of apomixy might be occurring. Further studies about embryology and biology of the reproduction of these plants are planned.

Conclusions

This study confirms the necessity to preserve the *P. crystallina* subsp. *hirtiflora* population from Rossano (Calabria, southern Italy) because it is the only Italian population which show $2n=27$, a cytological peculiarity known otherwise only from plants in Greece. The small habitat recreated in the Botanical Garden of Calabria University contributes to this aim and has also become a major point for guided visits, where the school students and visitors are spellbound and interested from this delicate and aesthetically attractive carnivorous plant.

Acknowledgements: Many thanks are due to the curators of FI, NAP and RO herbaria for the precious help and availability, and in particular to Dr. Annalisa Santangelo (University of Naples) for sending us digital pictures of original material; to Jessica Barbieri for helping in the traslation work.

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Figure 3. Full-view of the microhabitat where *P. crystallina* subsp. *hirtiflora* is cultivated in the Botanical Garden of Calabria University.

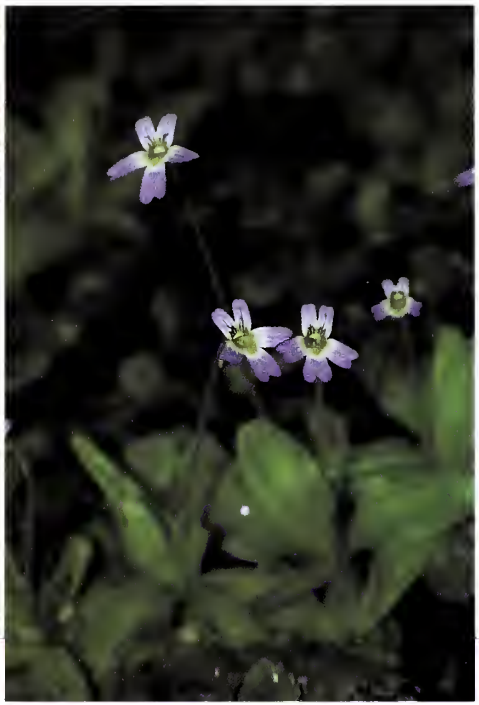

Figure 4. *P. crystallina* subsp. *hirtiflora* from Botanical Garden of Calabria University in full anthesis, general view.

Figure 5. *P. crystallina* subsp. *hirtiflora* from Botanical Garden of Calabria University in full anthesis, particular of the flower.

Figure 6. Seedlings of *P. crystallina* subsp. *hirtiflora* germinating in the Botanical Garden of Calabria University.

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News and Views

Michael S. Bodri and Alana Gaspard (bodrim@nsula.edu) write: While investigating the pollination biology of a population of *Sarracenia alata* in Louisiana, we noticed some obvious and striking differences in the petals of this species. Anthesis amongst the plants of our study site was approximately 2 weeks in duration, with some plants blooming weeks earlier and others weeks later than the majority of the population. Flowers of late blooming plants were frequently distorted, with "blasted" buds, eontorted flower heads, and lacking petals. We also observed a fair amount of variability in regards to petal color, from the typical yellowish-white, to pure white, slightly blushed with pink, to significantly blushed with red (see Figure 1, left). No other species of *Sarracenia* are growing naturally in the area, and the closest cultivated plants are more than 30 miles distant, so hybridization can be ruled out as a source of the pink or red petal coloration. *Pinguicula pumila*, *Utricularia subulata*, *Drosera brevifolia* and *D. intermedia* also occur at this site.

Figure 1: *Sarracenia alata* flowers in Louisiana. Photographs by Michael Bodri.

In addition to the color variability was the finding of a number of *Sarracenia alata* plants with cleft petals (see Figure 1, right). These plants were scattered throughout the population. The plants bearing cleft petalled-flowers displayed this characteristie in all their flowers, so it was not just a case of a few abnormalities created by frosts, disturbance, etc. As in the flower colors, variability of the degree of petal incision was noted, suggesting dilution of a genetic trait due to cross-pollination.

Stewart McPherson (61 Lake Drive, Hamworthy, Poole, Dorset BH15 4LR, Great Britain, stewart@mcpherson.net) writes: I am seeking members interested in joining a private expedition to Venezuela to survey and study species of *Heliamphora* in their wild habitat: the unearthly wind earved summits of the tepui plateaus. Transportation is by helicopter, starting from Santa Elena on the Brazilian boarder. In addition to *Heliamphora*, *Brocchinia*, *Utricularia* and *Drosera*, the tepuis offer perhaps the most incredible landscape on the planet. No previous mountaineering experienee required—just desire and determination to experience this extraordinary part of the world. If you are interested, contact me immediately.

Announcement!

As you may have noticed from this issue's inside cover, the ICPS has changed its business address to: ICPS, Inc., PMB 322, 1564-A Fitzgerald Drive,Pinole CA 94564-2229 USA.

Noteworthy Collections: Anthocyanin-Free *Sarracenia alata* (Wood) Wood in Alabama

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Keywords: field studies: Alabama (USA), pigmentation, *Sarracenia alata*.

Sarracenia alata (Wood) Wood is widely varied in color and morphology throughout its range. The expression of the red pigment anthocyanin creates many beautiful and very different color forms, from lightly veined to a solid maroon that is, in extreme cases, almost black.

Over the years, many anthocyanin-free forms of *Sarracenia* species and subspecies have been found in the wild, including *S. flava* (Bill Scholl, pers. comm.), *S. leucophylla* (Sheridan & Scholl, 1996), *S. minor* (Sheridan & Scholl, 1996), *S. psittacina* (Sheridan & Scholl, 1993), *S. purpurea* subsp *purpurea* (Case, 1956), *S. rosea* (=*S. purpurea* subsp. *purpurea* var. *burkii*) (Hanrahan & Miller, 1998) *S. rubra* subsp. *jonesii* (Case & Case, 1976), *S. rubra* subsp. *gulfensis* (Sheridan & Scholl, 1993) and a peculiar population of *S. rubra* subsp. *rubra* from western Georgia that has been suggested as being a possible ancestral progenitor for the *S. rubra* complex (Sheridan & Scholl, 1993). However, an anthocyanin-free form of *S. alata* has not previously been reported.

On August 20, 2001, we were botanizing in a seepage bog in southwestern Alabama (Washington County). The site housed various carnivorous species including *Sarracenia alata*, *S. leucophylla*, *S. psittacina*, and hybrids thereof, *Pinguicula lutea*, *Drosera capillaris*, and *Drosera filiformis* var. *tracyii*. Other field workers have reported *S. rubra* subsp. *wherryii* growing in this particular bog (Jay Lechtman, pers. comm.), however, it was not found during this expedition. While surveying this site we stumbled upon a single, large clump of *S. alata* that caught our attention because it was a distinctively uniform light green color, without the red venation that characterized all other specimens of this species at the site. We observed that there was a complete absence of anthocyanin in both the growth point and leaves of the plant (see Front Cover). A flower was not observed; however, we assume that it will exhibit the typical white/yellowish coloration of other anthocyanin-free forms of *Sarracenia*. Like other *S. alata* from southwestern Alabama, this specimen had a slightly pubescent exterior.

Many hours were spent searching the immediate area of the all-green *S. alata* for other specimens; none were found. Jay Lechtman visited the site again in the early spring of 2002—although the anthocyanin-free plant was relocated, an exhaustive search found no other anthocyanin-free *S. alata* plants (J. Lechtman, pers. comm.). To date, no other reports of anthocyanin-free *S. alata* have been published or otherwise publicized.

GPS coordinates for this clump were obtained and reported to the ICPS conservation program. This information will only be revealed to qualified conservation workers.

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A large-flowered form of *U. bisquamata* from near Hermanus, South Africa

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Keywords: observations: South Africa, *Utricularia bisquamata.*

Utricularia bisquamata is a widespread and variable bladderwort from southern Africa. During my travels in South Africa in 1997 and 1998 I observed a number of forms in the wild and from herbarium collections, on which this account is based.

Utricularia bisquamata is a taxon that has been described under several different names, including the more popular and recently rejected *U. capensis* (Taylor, 1989). Following examination of the eighty or so specimens housed at the Compton Herbarium, a noteworthy range in size of the flowering parts became apparent, even though the morphology of the traps and stolons in the specimens are identical (Taylor, 1989). The majority of specimens had yellow and purple flowers less than four mm across, whilst about twenty collections had flowers up to eight mm across, and only eleven collections had larger flowers.

From my observations of this species in the southwestern part of its range, the most widespread form has the smallest flowers. These are commonly purple and yellow, although forms with a yellow lower petal and yellow palate and upper petal are not uncommon (see Kondo & Kondo, 1983: page 157 for illustrations of different colour forms). It grows as a perennial in

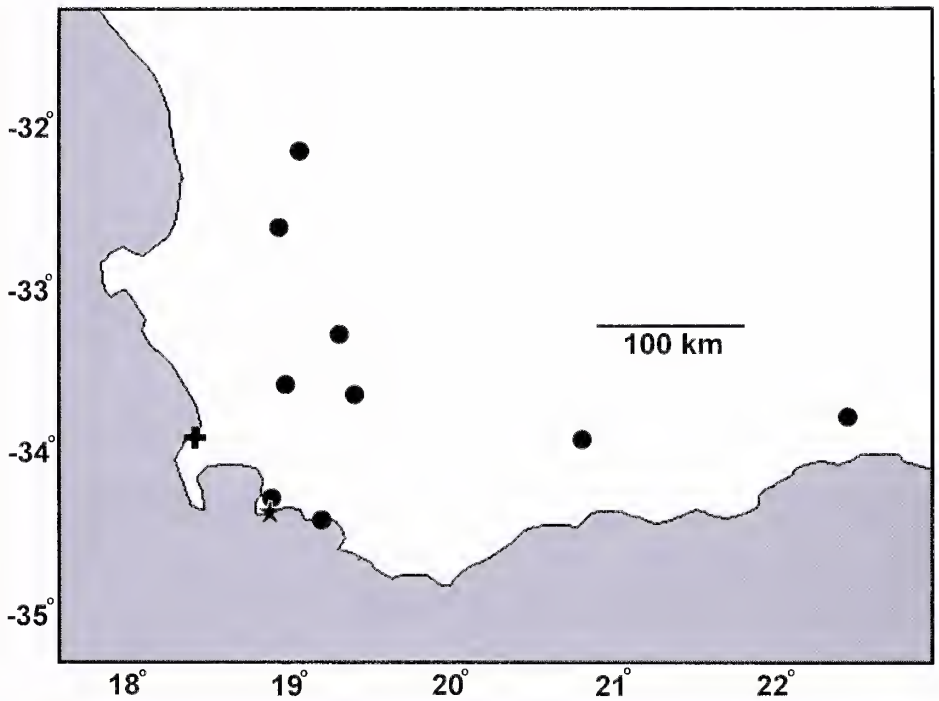

Figure 1: Distribution map of large flowered populations of *Utricularia bisquamata*, based on collections housed at the Compton Herbarium, Cape Town. Note the location of Betty's Bay, shown by the star; the location of probably the best-known large flowered form now in cultivation. The centre of Cape Town is indicated by the solid cross.

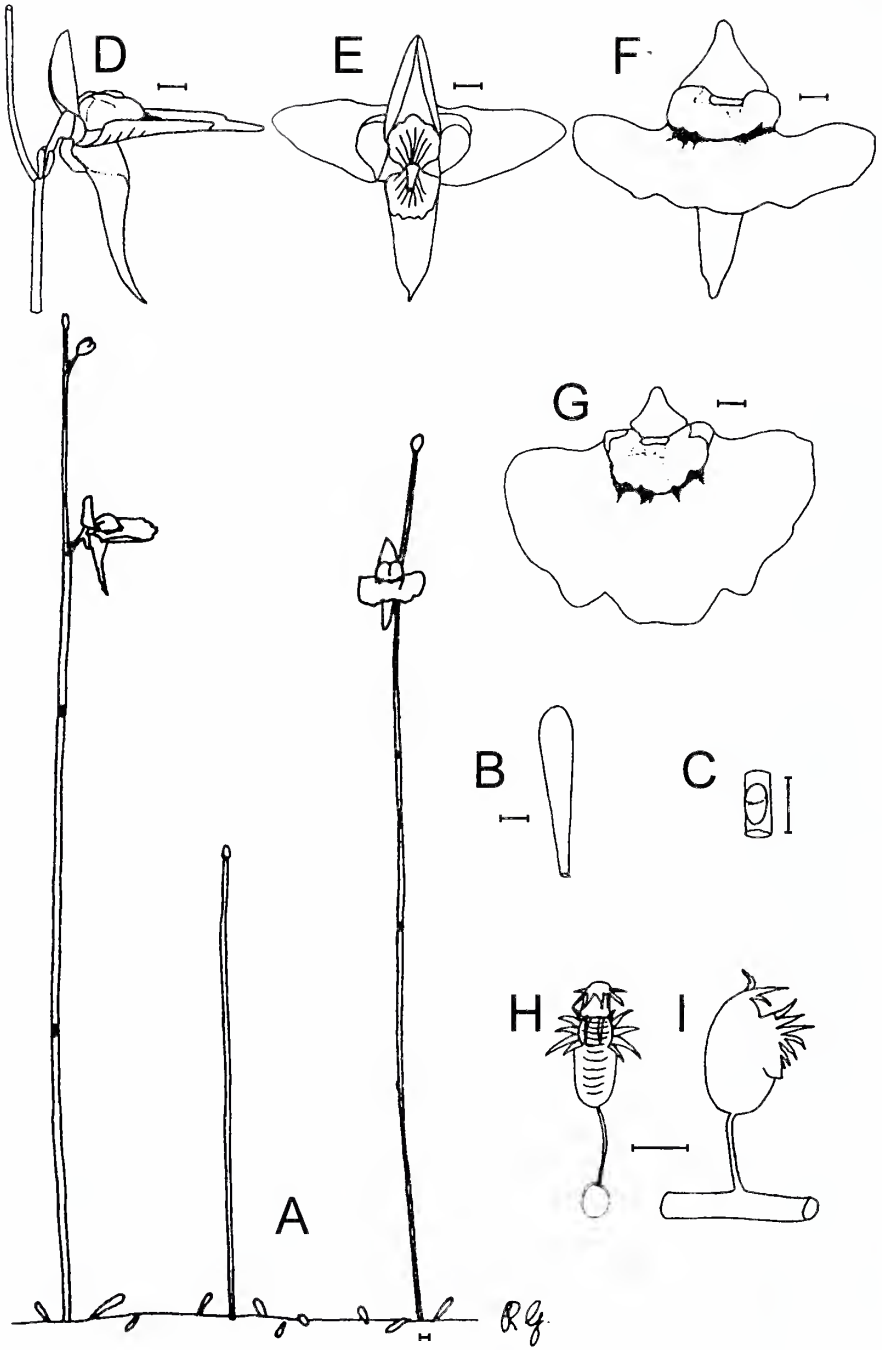

Figure 2: Botanic illustration of the large flowered form of *U. bisquamata* from near Hermanus grown by Eric Green. A: above ground group of plants; B: lamina; C: basi-fixed bracts; D: flower from the side; E: flower from the front; F: flower from above; G: flower from behind; H: trap from the front; I: trap from the side. Note the scale bars equal 1 mm for all plant parts except the traps (H and I) where they are 0.2 mm long. Illustrated by R. Gibson, 15-xii-1997.

permanent seeps and swamps, but appears to grow as an annual in thin, moss-covered soil beside small intermittent streams and on exposed seepage zones in the sandstone ranges in and around Cape Town. The annual form sets seed easily and appears to be able to self-pollinate its flowers. Forms with flowers of intermediate and large size are scattered throughout the range, but do not necessarily occur side by side. For example, the large flowered form of this species occurs in one swamp near Hermanus whilst plants of typical size occur in adjacent swamps.

Although I have not seen plants of intermediate flower size in the wild, there is a form becoming common in cultivation in Australia, which horticulturists have been calling *Utricularia* sp. "Bainskloof". This has flowers of similar colour to the typical form of the species, but has a lower petal of irregular semi-circular outline to about eight mm across, and thus appears to belong to the intermediate sized form. From the lack of seed set in cultivation, it appears to lack autogamous self-pollination.

The large flower form occurs in a number of locations in the southwestern Cape, as shown in Figure 1. Taylor (1989) reports similarly robust flower forms of this species from Angola and Madagascar. I had the pleasure of studying one population of this form from the Kleinriviersberge, near Hermanus, approximately 100 km south east of Cape Town. Plants in this population were in flower in December 1997 (see Figure 2, and Back Cover). Each scape grew up to 20 cm tall. The flowers had a transversely elliptic horizontal lower petal 7 mm long by 11 mm wide with a shallowly lobed free edge. The palate of the lower petal consists of a raised dome approximately two mm long by four mm wide which was yellow in colour and edged irregularly by dark yellow markings. The rest of the flower, including the small vertical upper petal, with reflexed margins, and sub-horizontal nectary spur, was lilac in colour. Overall this form of the species superficially resembled *U. dichotoma* more than the more typical form of this taxon.

This form of the species appears to have limited ability to self-pollinate its flowers and has more exacting cultivation needs than the typical, somewhat weedy form. In the wild at the study site this form grows in the company of *Drosera*, including *D. admirabilis, D. aliciae, D. capensis, D.* × *corinthiaca* (=*D. aliciae* × *D. glabripes*) and *D. slackii* (see Gibson, 2000, page 108), and would likely grow best in a peaty medium that is kept permanently wet and placed in a partially sunny position.

In conclusion, the large flowered forms of *U. bisquamata* offer novel and attractive forms for collectors. They may also provide a model for the study of different pollination and breeding systems for a variable species. and also provides an opportunity to study pollination and breeding systems of forms in a variable species.

Acknowledgements

I wish to thank Dr. John Rourke, and the helpful staff of the Compton Herbarium, Cape Town and Dr. Neville Marchant, of the Western Australian Herbarium for granting study access to the collections. I also wish to thank Eric Green, Frank Wolpert, Dot Cappaert, Yves-Andre Utz, Phil Wilson and Greg Bourke for their valuable and variable assistance in studying this taxon.

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Looking Back: CPN 25 years ago

Alain Godbout provided text from several letters Charles Darwin had sent to his companions. In a very interesting missive to Asa Gray (October 22, 1872), Darwin described a kind of acupuncture method he used on Venus flytraps: "The point which has interested me most is tracing the nerves which follow the vascular bundles. By a prick with a sharp lancet at a certain point, I can paralyse one half the leaf, so that a stimulus to the other half causes no movement. It is just like dividing the spinal marrow of a frog; no stimulus can be sent from the brain or anterior part of the spine to the hind legs: but if these latter are stimulated, they move by reflex action."

Observations on the *Aldrovanda*-containing and *Aldrovanda*-lacking Areas of Girraween Lagoon, Australia

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Keywords: observations: *Aldrovanda vesiculosa*, Northern Territory (Australia).

Observations of any plant in nature often help deciphering that plant's cultural requirements. Since questions often arise as to the best way to grow *Aldrovanda vesiculosa*, I am reporting my observations of the conditions at Girraween Lagoon just south of Darwin in the Northern Territory, Australia. This permanent lagoon is many hectares in size, and contains *Aldrovanda* to the right side of a boat launch, when facing the water (see Figure 1). On the other side of the boat launch is a similar area which does not contain *Aldrovanda*, even though it is only about 20 m away from the *Aldrovanda*-containing area. I hoped that observations of the two areas—both perhaps 50 m long—might help in understanding cultural preferences of *Aldrovanda*. These observations were made in midwinter, 25 June 2002. These plants are from a form which is not red year round but rather only during colder temperatures—it is the form of Australian *Aldrovanda* most commonly grown outside Australia.

The pH was measured using Baxter pH paper, the temperature was recorded using a meat thermometer inserted into the water (the accuracy of the thermometer was probably about ± 1°C). The plants, protists, and small animals such as crustaceans were examined by eye. Small samples of water were taken; these samples were studied microscopically and photographed within 6 hours. No material was permanently retained; rather some water containing algae was moved to a different site for easier use of the microscope.

The pH values in the *Aldrovanda*-containing and *Aldrovanda*-lacking sides of this site were simi-

Figure 1: The *Aldrovanda*-containing area at Girraween Lagoon. The white arrow points to shallow channel among sedges and rushes where *Aldrovanda vesiculosa* and *Utricularia muelleri* grow.

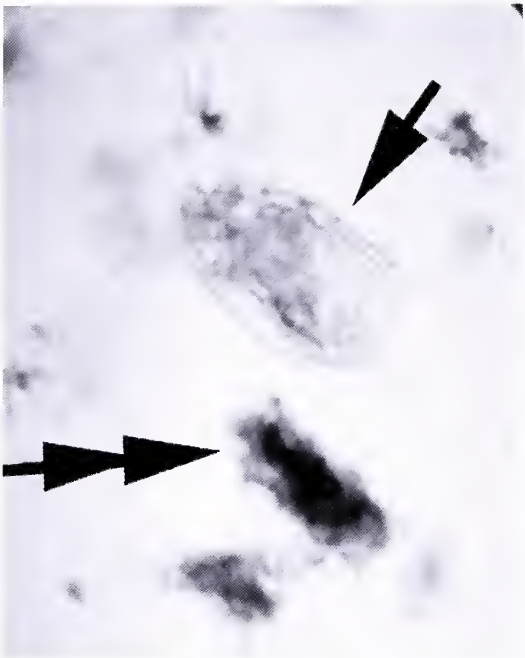

Figure 2: A micrograph of a sample from the *Aldrovanda*-containing side of the site. The single arrow points to a *Daphnia*-like crustacean and the double arrowhead points to a piece of organic debris. The *Daphnia*-like organism is about 0.5 mm long.

lar (Table 1), but both the level of incident sunlight and the temperature on the *Aldrovanda*-containing side were much higher because the tree canopy was more complete on the side lacking *Aldrovanda*. It is unclear whether allelopathy by the trees could explain the difference between the two sides—*e.g.* whether toxins from fallen leaves could have been killing any *Aldrovanda* plants which reached the *Aldrovanda*-lacking side. There was also much more non-carnivorous emergent vegetation on this side.

Microscope observations of the two sides of this site (three slides were made from each of the two sites) showed somewhat similar distributions of microscopic organisms, ranging in size from single cells to small crustaceans, although the size and numbers of the samples were not very extensive.

Another carnivorous plant, *Utricularia muelleri*, also grew on the *Aldrovanda*-containing side, while a non-carnivorous eukaryotic macroalga, a member of the stonewort algae or Characeae, grew on both sides along with smaller diameter eukaryotic string algae, although the string algae grew less vigorously on the *Aldrovanda*-containing side. Taken together, these observations suggest that the greater lighting on the *Aldrovanda*-containing side may be important for reducing the growth of string algae (which can harm *Aldrovanda*) and for increasing growth of emergent vegetation (with which *Aldrovanda* often grows well). Loosely organized cyanobacteria were also observed in the *Aldrovanda*-containing side—they are also a common occurrence with healthy *Aldrovanda* in cultivation. The presence of *Utricularia muelleri* and cyanobacteria on the *Aldrovanda*-containing side are indicative that this side of the boat launch was a healthier place for *Aldrovanda* to grow, as has been reported elsewhere (Darnowski, 2002).

Thus, it seems from these observations that the key difference between the *Aldrovanda*-containing side and the *Aldrovanda*-lacking side of the site at Girraween Lagoon may be difference in lighting and the different flora and fauna that accompany it. Given that the sites are connected and only about 20 meters apart, it is unlikely that significant differences in water chemistry would occur between them. The differences in lighting and in flora and fauna the most likely causes of the presence or absence of *Aldrovanda* on the two sides. These observations agree with observations of artificial habitats in which *Aldrovanda* grows successfully (Chadwick & Darnowski, 2002; Darnowski, 2002), further indicating

Table 1: Conditions on the *Aldrovanda*-containing and *Aldrovanda*-lacking sides of the boat launch.

Feature observed	*Aldrovanda*-Containing	*Aldrovanda*-Lacking
pH at shoreline	4.5-5.0	4.5-5.0
Water temperature (°C)	27.2	22.8
Amount of sedge and rush	Abundant	Sparse
Amount of light	Dappled, ample	Heavy shade
Utricularia muelleri	Present	Absent

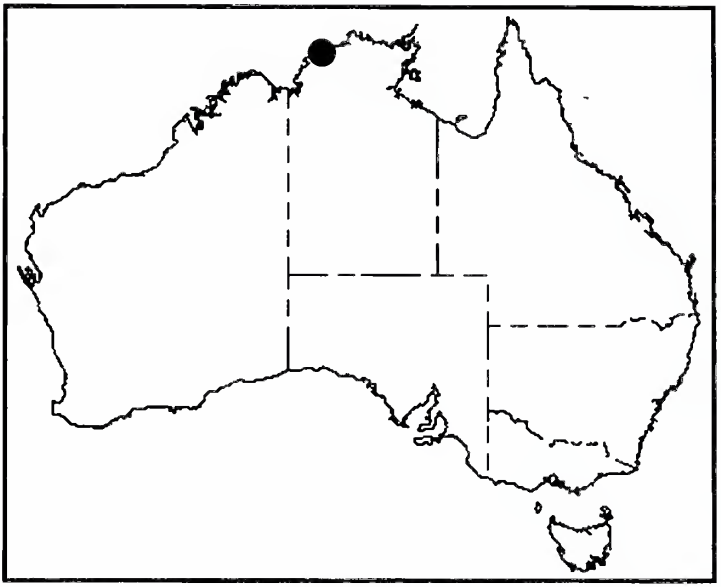

Figure 3: The location of Girraween Lagoon, immediately south of Darwin, Northern Territory, indicated with a dot.

the importance of the indicated conditions for the successful cultivation of *Aldrovanda vesiculosa*.

References:
Darnowski, D.W. 2002. A method for growing *Aldrovanda*. Carniv. Pl. Newslett. 31:113-115.
Chadwick, Z.D. and Darnowski, D.W. 2002. Observations on prey preference and other associations of *Aldrovanda vesiculosa* in a new culture system. Proceedings 4th International Plant Conference, Tokyo, Japan, pp. 39-47.

Table 2: Microscopic observations on samples from the *Aldrovanda*-containing and *Aldrovanda*-lacking sides of the boat launching site.

Algae, Diatoms, and Debris		
Feature observed	*Aldrovanda*-Containing	*Aldrovanda*-Lacking
Eukaryotic string algae	Present	Present
Characeae—little Ca crystals	Present	Present
Cyanobacteria	Loosely organized	With gelatinous sheath
Diatoms	Present	Absent
Organic debris	Present	Present
String algae	Present, not abundant	Present
Animals		
Daphnia-like organisms	Present	Present
Nematodes	Present	Present
Non-predatory Ciliates	Few	Many
Predatory Ciliates	Absent	Present
Arthropod larvae	Present	Absent
Ostracods	Present	Absent
Copepods	Absent	Present

New Cultivars

Keywords: cultivar: *Dionaea muscipula* 'Cupped Trap', *Drosera regia* 'Big Easy', *Drosera* 'Rhodesian Beauty', *Pinguicula* 'Enigma', *Sarracenia* 'Katerina', *Sarracenia* 'Sorrow'.

Sarracenia 'Katerina'

Submitted: 7 July 2003

The cultivar originated from a retrogressive pollination of an F1 *Sarracenia* × *mitchelliana* (=*S. leucophylla* × *S. purpurea*) with *S. leucophylla.* I performed this cross in 1996 at the Botanic Gardens Liberec; out of many seedlings, the most purple and stiffly erect specimen was selected (Figure 1).

The pitchers of *Sarracenia* 'Katerina' are erect, around 60 cm high (24 inches, including the lid), with a mouth 5.5 cm (2 inches) wide. With the exception of the base, mature pitchers are entirely purple with red venation. The venation is particularly apparent in young pitchers. Mature pitchers have a spectacular appearance because of the translucent tissues (Figure 2).

In general form and habit, the pitcher lids are very similar to those of *S. leucophylla.* The sinuous (but undivided) margins of the lids are particularly ornamental. The lid's upper surface is densely hispid with very short (0.1 mm) purple hairs, and it is therefore velvety. The lower lid surface is glossy, covered with 2.2-2.5 mm long deflexed transparent bristles. The pitcher tube is externally only scarcely pilose. The spring pitchers and summer pitchers (which last over the winter) are very similar; the primary difference being that the spring pitchers have somewhat more green on the basal part of the pitchers. The rhizome is horizontal, like in *S. leucophylla.* Flowers are purple, intermediate between those of *S. purpurea* and *S. leucophylla.* The peduncles are long, like in *S. leucophylla.*

This cultivar should be propagated vegetatively to maintain its characteristics. Fortunately it is very vital and can be easily propagated by rhizome cuttings.

The original cultivar name (*Sarracenia* 'Katerina') is in Czech language, and honors Miss Katerina Stará, who manages the permanent exhibition of carnivorous plants in the Botanic Gardens Liberec (www.botanickazahradaliberec.ez) in the Czech Republic. The English translation of the name is 'Catherine', so according to the article 32 of ICNCP (1980), *Sarracenia* 'Catherine' can also be used when referring to the cultivar.

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Sarracenia 'Sorrow'

Submitted: 7 July 2003

We obtained a large number of *Sarracenia purpurea* subsp. *purpurea* seeds from a commercial firm in Miami, USA. These were sown in 1979 at the Botanic Gardens Liberec, Czech Republic. Among the various resulting seedlings, I observed a partially albinotic mutant plant and selected it for further observation.

In morphology, the plant is identical to normal *Sarracenia purpurea* subsp. *purpurea* typically encountered in the wild and in collections, but there are spectacular differences in its pigmentation. The pitchers are pale green (in strong sun, rather yellowish), with a prominent cross-like purple sign on the inner surface of the lid, just over the pitcher opening (Figure 3). Flowers are creamy white, in juvenile stage faintly pinkish (Figure 4). Despite these peculiar characteristics, I do not believe the plant is a hybrid.

In order to maintain this plant's unique characteristics, it should be propagated vegetatively. Unfortunately, typical of this species the plant is slow to propagate by divisions. Because of this, *Sarracenia* 'Sorrow' is probably of interest to enthusiastic *Sarracenia* collectors only.

In naming the cultivar with the epithet 'Sorrow', I remember my tragically lost son Miloslav (1975-1997). Perhaps the blood-red cross on the pitchers and the pure colour of flowers are my inspirations to give this plant such an unusual name.

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Dionaea muscipula 'Cupped Trap'

Submitted: 29 July 2003

This clone of *Dionaea muscipula* was first isolated by the staff at Agri-Starts III, Inc (19825 S.R. 44, Eustis, FL 32736 USA) in 1998. It forms traps that are distinct from all other cultivars of this species, in that the traps are fused at the distal end, forming a cupped appearance when the trap is open. Because of this strange form of the traps, the plant is being named *Dionaea muscipula* 'Cupped Trap.' The trap cilia on the margins retain their coiled position until after the traps open, displaying the circinate growth form. Despite these mutations, the traps still effectively capture insects.

The leaf blades of *Dionaea muscipula* 'Cupped Trap' are also unique in their obovate shape and serrulate margins.

Dionaea muscipula 'Cupped Trap' is somewhat difficult to grow to large size due to its abundant rhizome division and asexual formation of plants on inflorescence. The specimen pictured (Figure 5) is approximately 9.5 cm (3.75 inches) in diameter with traps 2 cm (0.75 inches) in length.

Propagation should be by vegetative means.

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Drosera regia 'Big Easy'

Submitted: 16 September 2003

In June of 2000 I attended the 2000 ICPS conference at the Fort Mason Center in San Francisco, California. While there I purchased a small *Drosera regia* plant from the vendor, "Marie's Orchids." The label identified it as "REG-1," but I have never discovered the significance of this marking on the label.

Many times during the better part of three decades I have germinated seed of *Drosera regia* with great success. Success, that is, in germination only, because invariably within a few weeks the seedlings would die for reasons unknown. I obtained this plant with just the same expectations, any confidence in my ability to successfully grow *Drosera regia* had been lost long ago with the lives of those many seedlings and from the stories told by those few who had reported success with this plant. Their complicated tales of successful cultivation made it sound like *Drosera regia* was the *Drosera* from hell, at least that's how it seemed to me then.

Soon, I was to discover that my former experiences with *Drosera regia* would be distant, fading memories. Under my cultural conditions this clone *Drosera regia* 'Big Easy' has been just that, big and easy to grow and propagate. It produces leaves a maximum of 23 cm (9 inches) long (Figure 6). It has never gone dormant, never flowered or even initiated a flower stalk. Several times I have managed to propagate it from leaf cuttings floating in jars of pure water (see detail in Figure 3). It readily initiates buds and plants on any and all pieces of severed root. I have propagated hundreds of plants this way and have traded them widely.

Other than its ease of cultivation, which is its most distinetive characteristic, its compact size is a distinctive characteristic of this cultivar.

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Pinguicula 'Enigma'

Submitted: 29 September 2003

In the 1990s, Carl Schoenfeld of Yucca Do Nursery, Inc.[1] and John G. Fairey of Peckerwood Garden collected a group of *Pinguicula* plants on one of their plant hunting trips to Mexico. Carl had previously met Ron Gagliardo from Atlanta Botanical Garden and was very impressed with their carnivorous plant collection. Carl chose to provide these plants to Ron for inclusion in the Atlanta Botanical

Figure 1: *Sarracenia* 'Katerina'. Photograph by M. Studnicka.

Figure 2: *Sarracenia* 'Katerina'. Photograph by M. Studnicka.

Figure 3: *Sarracenia* 'Sorrow'. Photograph by M. Studnicka.

Figure 4: *Sarracenia* 'Sorrow'. Photograph by M. Studnicka.

Figure 5: *Dionaea muscipula* 'Cupped Trap'. Left: plant in cultivation; top right: developing trap with circinate marginal spines; bottom right: closer view of mutated trap. Photographs by Steven Stewart.

Figure 6: *Drosera regia* 'Big Easy'. Photograph by William Joseph Clemens.

Figure 7: *Pinguicula 'Enigma'*; summer foliage in inset. Photographs by Travis Wyman.

Figure 8: *Drosera* 'Rhodesian Beauty'. Photograph by William Dawnstar.

Gardens collections. In August of 2002, Ron supplied me with specimens of these plants and asked for my help in distributing them to the carnivorous plant grower community and assisting in determining their identification. For further information on the Yucca Do-related *Pinguicula*, refer to Eric Partrat's web site[2].

With the acknowledgment of both Carl and Ron, I have chosen to give the cultivar name *Pinguicula* 'Enigma' to the plant labeled as ABG 97-1715 Yucca Do 60-45-031395B (see Figure 7). Its spoon-like leaves with upturned margins characterize the carnivorous foliage of this plant. During this phase of growth the rosette consists of up to twenty leaves, which are up to 4 cm long and 1 cm wide at their widest point. Initially the leaves tend to be held at an upright angle, but later they arc down to lay flat. The leaves remain a light green colour, even under intense light. During succulent growth, the size of the rosette decreases and the number of leaves increases to 50 or more. These leaves lack any upturned margins and are covered with sparse fine hair. They are spathulate in shape and up to 2 cm long and 0.75 cm wide. The plants bloom from early spring through summer. Flowers generally occur in pairs and it is not uncommon to have 2-3 pairs open at the same time. The colour of the flowers is lilac with very prominent violet veins and a white throat, and they often have a velvety sheen to them. As with many of the Mexican *Pinguicula* the colour tends to fade slowly as the flowers age. The back of the flower is pale-grey/white in colour and the veining is also prominent. Fully open flowers typically measure 2.5-3 cm in diameter. This plant is easily propagated via leaf cuttings and also has a tendency to divide readily after flowering, forming large clusters. The identity of this plant is unknown but I believe it to be a naturally occurring hybrid between two other plants in the Yucca Do-related *Pinguicula*; ABG 97-1714 Yucca Do 60-45-031395A which appears to be *P. cyclosecta* and ABG 97-1717 Yucca Do 60-45-031395C which is very similar in appearance to *P. esseriana* or *P. jaumavensis*

Web sites cited in text:

[1]http://www.yuccado.com

[2]A World of *Pinguicula*, http://perso.club-internet.fr/cpartrat/Pages/yucca_do_complex.htm

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Submitted: 18 December 2003

This beautiful plant has numerous leaves and tentacles on a short stem(see Figure 8). It retains each leaf for a long time, giving the plant a lush appearance. The petioles of the plant widen gradually and uniformly in a narrowly spathulate fashion. In good light the plant is a uniform golden green, with deeply red-colored glands. Lower light conditions will result in plants that are a deeper green, although the glands will stay red maintained. Although only the top three layers of leaves have active dew production, the lower layers of leaves retain their red and green coloration well.

The plant was originally sent to me for consideration under an incorrect name. I sent inquiries to *Drosera* field experts, taxonomists and horticulturists, and their consensus opinion was that the plant was a South African native. Furthermore, they generally felt it was likely to be a hybrid, but further clarification as to the plant's identity was uncertain. Many felt that this was identical to a form that has been widely sold by nurseries, and subsequently traded by growers, under the bogus name of *Drosera* "sp. Rhodesia". Since I believe this is likely, I chose the name *Drosera* 'Rhodesian Beauty' to closely match the previous name as much as possible. Propagation is best done vegetatively. I have had the best fortune using leaf cuttings.

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How to Grow a Ridiculously Large Number of Sundews

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Keywords: cultivation: *Drosera*, tissue culture.

Introduction

Let's face it: carnivorous plant people are a little nutty about their plants, and I am an excellent example. And if you are nutty about one single pygmy sundew or lowland *Nepenthes* in your collection, wouldn't it be even better to have fifty? Of course it would! So how do you propagate and maintain that many? Seeds and cuttings are an excellent way to multiply plants, but keeping many copies, of many species, happy in a greenhouse or terrarium is beyond the reach of most enthusiasts' growing space. Add to that the difficulties from some species of carnivorous plants that just do not reproduce easily—for example *Drosophyllum* or *Roridula*.

Tissue culture can solve many of these problems. Although the technique probably scares away many amateur growers who imagine that a prohibitive amounts of money, space, and expertise are required to do it, this is not a fair prejudice! Tissue culture is a relatively straightforward way to multiply plants, and some carnivorous plants will even maintain their normal morphology and annual cycle, to the point of flowering, *in vitro*. That term, *in vitro*, means "in glass," and originated when the early practitioners used glass containers. Many still do, although plastic dishes are also common.

Tissue culture is also a tool for conservation! By rapidly multiplying rare species, tissue culture helps remove pressure from natural populations—widely available, inexpensive plants propagated from tissue culture offset the incentive to poachers. Also, plants from tissue culture are often more vigorous than seed-raised plants of the same species, perhaps because of the optimal conditions given to them when young, especially with regard to mineral nutrition.

A Brief History of Plant Tissue Culture

Tissue culture began sometime in the late 19th or early 20th century, depending on how strictly "tissue culture" is defined, as a means for testing the growth requirements of plants—if a plant could be isolated from the soil the importance of inorganic compounds such as iron, and organic compounds such as vitamins, could be studied with scientific precision. For example, by withholding a partieular substance, any developmental defects in the plants could be observed.

In vitro cultivation of plants has also played a large role in the study of plant development. These studies expanded knowledge of what plants parts can be used for cloning. White and Knudsen were two early tissue culture practitioners (ca. 1930-1960) who furthered our knowledge of growth requirements and developmental biology; some of their recipes for plant tissue culture media are still used even today.

Today tissue culture is used for a wide variety of applications. Many agricultural laboratories use a variety of tissue culture techniques. For example, all transgenic crops originated in tissue culture. Furthermore, tissue culture is still used in its traditional role in studies of growth requirements and of development.

A number of national and international societies are dedicated to the science of botanical tissue culture, including the International Association for Plant Tissue Culture and Biotechnology and the Society for In Vitro Biology, both of which are easily found on the internet.

Basics of Tissue Culture

"Aseptic technique" refers to the removal of all the microorganisms, bacteria, fungi, and protists from the surface of material to be grown in tissue culture. Aseptic techniques are necessary in tissue culture because of the rich growing media used. Plant tissue culture media are very rich because they are intended to last for relatively long periods, and any microbes entering the eultures quickly overwhelm the plants being cultured. A special challenge in plant tissue culture, as opposed to laboratory culture of

bacterial or other eukaryotic cell or tissue cultures, comes from the inability to add antibiotics to plant tissue cultures. The antibiotics used for killing most microbes in culture harm plant cells. They are also quite expensive. A new compound called Plant Preservative Mixture (PPM)[1] suppresses the growth of microbes, although some reports in the scientific literature say that PPM can cause abnormalities in plants. Since PPM only suppresses the growth of microorganisms (and does not kill them) a culture grown using PPM must always use the compound, or else the microorganisms will resume growth and overwhelm the plant cells.

All of the water that comes into contact with specimens used in tissue culture work needs to be sterilized to kill any microbes in it. Sterile water can be purchased from some lab suppliers but is very expensive. Use an autoclave or a pressure cooker made for cooking to sterilize water. An autoclave is essentially a larger lab-grade pressure cooker. Some smaller autoclaves may be in the price range of home users and can be plugged into a home outlet.

Ideally, a specimen of plant material to be used in tissue culture work is no more than a cm or two in all dimensions (and is often much smaller). Before it can be placed on sterile media, it is first surface sterilized (made aseptic on its surface) usually by using a dilute mixture of bleach (NaOCl, sodium hypochlorite), or a close chemical relative such as CaOCl (calcium hypochlorite)[2]. To make this sterilizing solution, household bleach is diluted with sterile water to 5-40% concentration. (Since the bleach concentration was originally only about 10% at the time of purchase, the additional dilution reduces the concentration to about 0.5-4%.) Tween 20 (polyoxyethylene sorbitan monolaurate, in syrup form) is added to the sterilizing solution to make a detergent concentration of about 0.1-1% by volume. The detergent acts as a wetting agent and allows the bleach to better access nooks and crannies in the plant material where bacteria and fungi may be hiding. The plant material is then rinsed with sterile water to remove the bleach solution, which would otherwise eventually kill the sample.

With luck, the plant specimen is now aseptic, and can be placed on tissue culture media. There are many formulations of media, but they all include a number of nutrients needed by plants: inorganic salts of macronutrients and micronutrients, and organic compounds such as vitamins and *myo*-inositiol. Sugar (usually sucrose) is included at the rate of about 3% w/v (weight/volume; 1ml=1g; so 30 g of sucrose is dissolved into 1000 ml of medium). Most media are designed to have a pH of 5.8, and are autoclaved to destroy any microbes or spores. The components of the medium survive the pressures and temperatures applied in the autoclave, although some media are filter sterilized instead, to better preserve the organic components of the media. These media will not be discussed further since they are expensive and impractical for home use.

Many media formulations have been devised over the years, and they are often specialized for particular types of plants. The three best-known formulations are called "Murashige and Skoog," "Linsmaier and Skoog," and "B5" (which is also called "Gamborg's Medium"). Combinations of the mixtures can be purchased from many supply houses. For example, MS/B5 is a dry ingredient formulation which has the macro and micro salts from the MS medium, and the B vitamins from the Gamborg *et al.* medium).

All these media should all be suitable for culturing carnivorous plants, although since carnivorous plants prefer lower rates of nutrients the media should be diluted to 5-20% normal concentration.

Instead of being fluid, media used in aseptic culture are solid. This is because most plants, including most carnivorous plants, prefer to grow upon a solid substrate (obviously, this is not necessary for *Aldrovanda vesiculosa* and aquatic *Utricularia*). Since the mixture of nutrients used in tissue culture is in a liquid form at room temperatures, it must be gelled into a solid form. Agar, often sold as Bactoagar is the compound most commonly used to effect this change[3]. The agar is usually used at 0.5-1% (w/v), and must be dissolved by heating the media to near boiling temperatures either during or before autoclaving. Conventional cotton balls are sometimes used as an alternative to agar. While it is cheaper and more easily available than agar, plants may be harder to disentangle from this solid support.

The medium (including the agar) is placed in a glass jar. Many plastic containers are rated for autoclaving, such as medical containers used to collect urine samples (hey, you have got to be creative). The containers are sealed using either a waxy tape-like material called parafilm or a breathable dressing,

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[1]This is available for purchase on the internet.

[2]Sometimes this step is preceded by briefly rinsing the plant tissue in 70% ethanol.

[3]While agar is by far the easiest gelling agent for the home user to obtain, others are also available but are not discussed here. Systems based upon using porous solid supports and liquid media are sometimes used, and the interested reader may wish to investigate these more.

available from most pharmacists in the surgical supplies or first aid sections (Figure 1). These reduce the loss of water from the cultures. This is important because it extends the lifespan of a culture, thus minimizing how frequently you must transfer the plants to fresh media.

Table 1: A Sample Schedule for Tissue Culture of a carnivorous plant	
Event	Time
Sterilize seed or stem, place in culture.	0
Culture begins to germinate/grow.	1-3 weeks
Complete plants develop.	2-4 weeks
Plants begin to crowd container.	4-12 weeks
Subculture required or culture lost.	4-20 weeks

All the necessary media ingredients can be obtained from Sigma Chemical or a companies which provide supplies to schools for science classes—*e.g.* in the USA, Carolina Biologicals or Ward's Natural Science. A simple search of the web will turn up many such sources with moderately priced components (see Table 2). One savings involves the use of table sugar in place of scientific-grade sucrose, a perfectly acceptable choice for home use. Some media can be bought already autoclaved and ready to use, which can be a big time and trouble saver.

I use a mixture of 20% MS/B5, 3% sucrose, and 0.8% Bactoagar (which has a pH=5.8 before autoclaving). This medium has been successful with *Drosera* of all types, *Sarracenia*, *Utricularia*, *Cephalotus*, *Genlisea*, *Heliamphora*, *Pinguicula*, and *Stylidium*. Most other carnivorous genera should do well on this same medium.

Setting up Tissue Culture at Home

To set up a home laboratory to do tissue culture, you will need a place with little traffic. Even small air currents are undesirable because they can introduce microbes and their spores, which could contaminate your media. In addition, some sort of "hood" is needed to further reduce contamination. The simplest and least expensive such device is a wooden box with a viewing window and holes in the side for inserting your arms. Protective gloves can be awkward to use and difficult to decontaminate, so bare hands and arms (with all jewelry removed), and skin wiped with 70% ethanol is the best procedure. This box can be equipped with an ultraviolet lamp—usually a fluorescent bulb of at least 60 cm (2 feet) in length for greater coverage of the work area. Microbes and spores inside the box will be killed by about 30-60 minutes of irradiation. You should not work while the ultraviolet lamp is operating.

A more effective (but also more expensive) alternative to such a box is a laminar flow hood. The laminar flow hood passes a constant flow of filter-sterilized air over cultures, ensuring that microbes do not waft into containers and spoil cultures (see Figure 2).

Both a wooden box and the laminar flow hood can be purchased from the same sources that provide tissue culture media. Wooden boxes generally cost about US$150, while laminar flow hoods cost ten times as much. You can save money by building your box and hood—plans can be found on the internet or in books on tissue culture. Older books are a particularly good source of construction plans, and you can often find these in most large libraries.

Autoclaves are not hard to find and not too expensive, although a cheaper alternative you may already own is a pressure cooker used for canning—the same pressures and temperatures which kill microbes in preserved food will kill microbes in tissue culture media.

You will require tools. Scalpels and forceps like those sold on campus supply stores for college dissection courses are just fine. At least one pair of long forceps, at least 15 cm (6 inches) long, is a great help when working with some types of culture vessels.

You will require a means of sterilizing tools, and keeping them sterile while you are using them. Tools are sterilized by first dipping them in a container filled with 95-100% alcohol, and then heating them in either an alcohol lamp flame or a heated sterilizing device containing either a ceramic cylinder or a heated container of glass beads. An alcohol lamp flame will cost US$10, the heated sterilizing device will cost US$75 or more and will require use of an electric outlet.

Once cultures have been established, they will need lights. Even though the sucrose in the media will provide all the sugars the plants will need, so they do not need to photosynthesize, light will promote the formation of normal plant structures. Either standard fluorescent bulbs or grow-bulbs may be used, and they should be turned on for 12-24 hours each day. Be sure to place cultures no more than 15

Figure 1: A tissue culture (*Stylidium linear*e) in a urine specimen container, in front of tissue cultures growing in smaller, 50 ml centrifuge tubes.

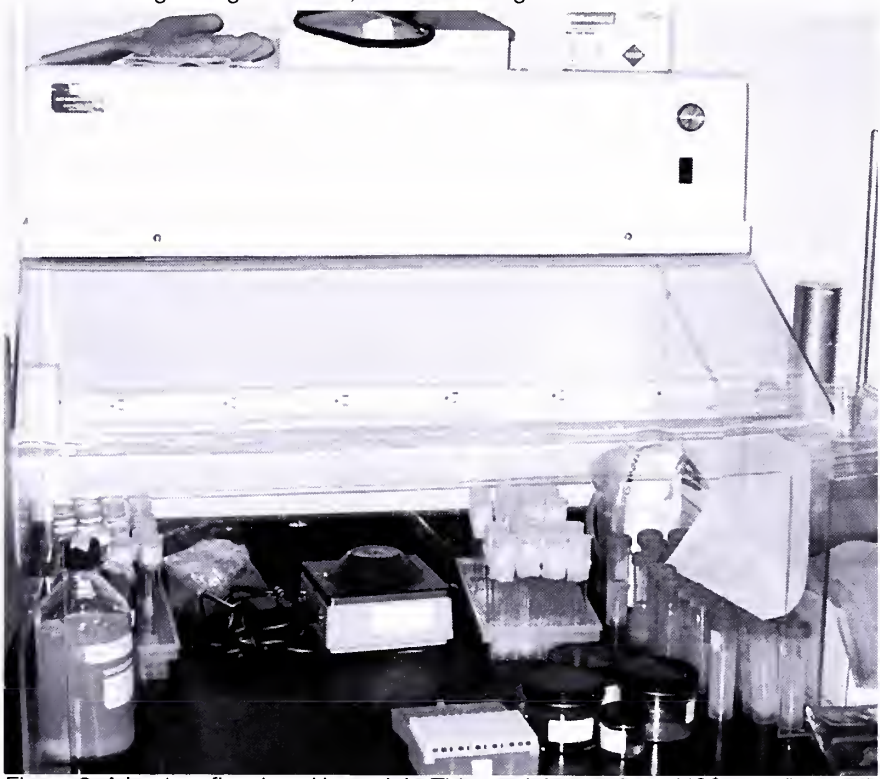

Figure 2: A laminar flow hood in my lab. This model cost about US$1500 (in 2003) and is about 1.3 m wide by 0.8 m deep by 1 m high (4 ft by 2.5 ft by 3 ft).

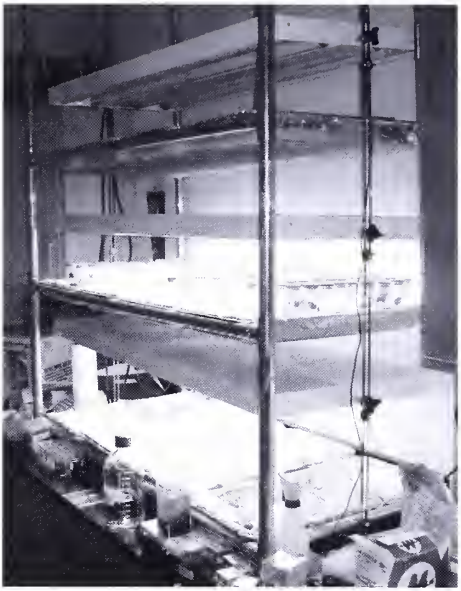

Figure 3: My light stand for tissue cultures. Only two shelves are in use in the picture. The stand is about 2 m high by 1.7 m wide by 0.7 m deep (6 ft by 5 ft by 2 ft).

Figure 4: A tissue culture of a pygmy sundew, with the fine roots visible against the side of the container.

cm (6 inches) from the lights for best results. The lights will generate significant amounts of heat, so temperatures in the culture vessels will be higher than ambient temperatures when the lights are lit. You should choose the temperatures that best match the growth requirements of your plants. While 23°C (75°F) is a good, general choice, some *Nepenthes* might grow better with higher temperatures while *Darlingtonia* might do better with less heat.

Light stands are constructed to support both your lights and your tissue culture media (see Figure 2). While light stands are commercially available, many carnivorous plant growers build their own. Reflective foil is used on the light stand supports, floors, walls, etc., to reflect as much light as possible to the cultures.

Once plants in culture are large enough, they can be removed from the sterile culture and grown on soil (Figure 4). This process is known as deflasking since cultures are sometimes grown in large flasks. Use standard soil mixes, but be sure to keep humidity extremely high for freshly deflasked plants. This is necessary because the humidity is very high inside culture containers, and sudden decreases in humidity may overstress and kill plants. You can keep the humidity high by enclosing the newly potted plants in a plastic bag or similar cover. The plant is hardened to lower humidity gradually, either by opening the enclosure by successive small amounts over a week or two, or by removing the plant from the cover completely for short (but increasingly long) amounts of time each day.

Using these methods, you will soon have more plants than you know what to do with, which when you think about it is a good thing.

Two excellent and recent books on tissue culture are listed in Table 2 as references. Many other older books also exist in libraries and might be better sources of plans for home users.

Table 2: Useful resources.

Web Sites
Sigma Chemical: http://www.sigmaaldrich.com/Brands/Sigma.html
Carolina Biologicals: http://www.carolina.com/
Ward's Natural Science: http://www.wardsci.com/
Kitchen Culture Kits: http://www.kitchenculturekit.com

Books
Roberta, H. 2000, Smith Plant Tissue Culture Techniques and Experiments, 2nd edition Academic Press, New York.
Trigiano, R.N., and Gray, D.J. (editors) 2000, Plant Tissue Culture Concepts and Laboratory Exercises 2nd ed. CRC Press, New York.

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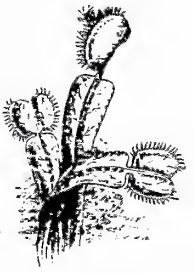

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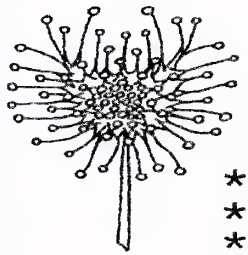

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